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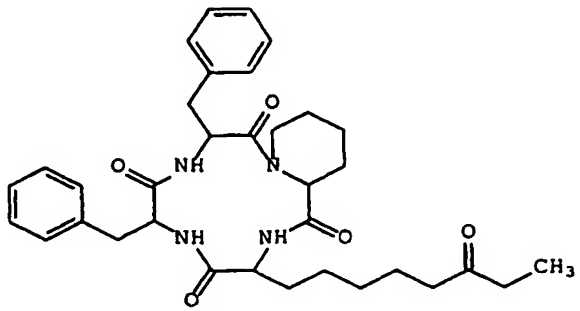

(I)

(57) Abstract

The present invention provides a new compound of formula (I) which has a histone deacetylase inhibiting activity, and a process for production thereof. Also provided is a pharmaceutical composition containing the compound for treating or preventing organ transplant rejections, autoimmune diseases, tumors, and the like.

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DESCRIPTION

INHIBITOR OF HISTONE DEACETYLASE

TECHNICAL FIELD

The present invention relates to a novel cyclic tetrapeptide compound, hereinafter entitled FR225497 substance which is useful as a medicament, to process for producing the same and to pharmaceutical composition comprising the same.

BACKGROUND ART

European Patent Application of EP0406725 discloses that Helicoma ambiens RF-1023 (FERM BP-2751) produces RF-1023-A (Trapoxin A) and RF-1023-B (Trapoxin B).

DISCLOSURE OF INVENTION

The present invention relates to a novel cyclic tetrapeptide compound, FR225497 substance.

More particularly, it relates to novel cyclic tetrapeptide compound, FR225497 substance, which is obtained from the culture broth of Helicoma ambiens RF-1023-1. In this respect, Helicoma ambiens RF-1023-1 is subculture of Helicoma ambiens RF-1023 (FERM BP-2751).

Now, we found FR225497 substance which is structurally related compound with RF-1023-A (Trapoxin A) and RF-1023-B (Trapoxin B). Compared with Trapoxin A and Trapoxin B, the FR225497 substance is markedly stable at the incubation with rat liver S-9 fraction. Therefore, FR225497 substance is considered to be strongly more effective than Trapoxin A or Trapoxin B.

The FR225497 substance has potent inhibitory effect on activity of histone deacetylase. Histone deacetylases are known to play an essential role

in the transcriptional machinery for regulating gene expression, and histone deacetylase inhibitors induce histone hyperacetylation and affect the gene expression. Therefore, histone deacetylase inhibitor, such as the FR225497 substance, is considered to be useful as a therapeutical or prophylactic agent for several diseases caused by abnormal gene expression such as inflammatory disorders, diabetes, diabetic complications, homozygous thalassemia, fibrosis, cirrhosis, acute promyelocytic leukaemia (APL), protozoal infection, or the like.

We also found histone deacetylase inhibitor, such as the FR225497 substance, has potent immunosuppressive effect and potent antitumor effect. Therefore, histone deacetylase inhibitor, such as FR225497 substance, is useful for an active ingredient of an immunosuppressant and an antitumor agent and useful as a therapeutical or prophylactic agent for an organ transplant rejection, autoimmune diseases, tumor, or the like.

Accordingly, one object of this invention is to provide a novel compound FR225497 substance which has biological activities stated above.

Another object of this invention is to provide a process for production of FR225497 substance by fermentation of a FR225497 substance-producing strain belonging to the Helicoma in a nutrient medium.

A further object of this invention is to provide a pharmaceutical composition containing, as an active ingredient, the FR225497 substance.

Still further object of this invention is to provide a use of the histone deacetylase inhibitors, such as FR225497 substance, for treating and preventing diseases stated above.

The FR225497 substance can be produced by fermentation of the FR225497 substance-producing strain belonging to the Helicoma such as Helicoma ambiens RF-1023-1.

It is to be understood that the production of the FR225497 substance is not limited to the use of the particular organism described herein, which is given for the illustrative purpose only. This invention also includes the use of any mutants which are capable of producing the FR225497 substance including natural mutants as well as artificial mutants which can be produced from the described organism by conventional means such as irradiation of X-ray, ultra-

violet radiation, treatment with N-methyl-N'-nitro-N-nitrosoguanidine, 2-aminopurine, and the like.

This strain was deposited in National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology (1-3, Higashi 1-chome, Tsukuba-shi, Ibaraki, Japan) as Helicoma ambiens RF-1023-1 (FERM BP-6413 (deposition date: July 9, 1998)).

Production of the FR225497 substance

The FR225497 substance is produced when the FR225497 substance-producing strain belonging the Helicoma is grown in a nutrient medium containing sources of assimilable carbon and nitrogen under aerobic conditions (e. g. shaking culture, submerged culture, etc.). .

The preferred sources of carbon in the nutrient medium are carbohydrates such as glucose, sucrose, starch, fructose or glycerin, or the like.

The preferred sources of nitrogen are peanut powder, yeast extract, beef extract, peptone, polypeptone, gluten meal, cotton seed flour, soybean powder, soybean meal, corn steep liquor, dried yeast, wheat germ, etc., as well as inorganic and organic nitrogen compounds such as ammonium salts (e. g. ammonium nitrate, ammonium sulfate, ammonium phosphate, etc.), urea or amino acid, or the like.

The carbon and nitrogen sources, though advantageously employed in combination, need not to be used in their pure form because less pure materials, which contain traces of growth factors and considerable quantities of mineral nutrients, are also suitable for use.

When desired, there may be added to the medium mineral salts such as sodium or calcium carbonate, sodium or potassium phosphate, sodium or potassium chloride, sodium or potassium iodide, magnesium salts, copper salts, zinc salts, iron salts, or cobalt salts, or the like.

If necessary, especially when the culture medium foams seriously a defoaming agent, such as liquid paraffin, fatty oil, plant oil, mineral oil or silicone, or the like may be added.

Agitation and aeration of the culture mixture may be accomplished in a

variety of ways, such as agitation by a propeller or similar mechanical agitation equipment, by revolving or shaking the fermenter, and the like.

The fermentation is usually conducted at a temperature between about 10°C and 40°C, preferably 20°C to 30°C, for a period of about 50 hours to 150 hours, which may be varied according to fermentation conditions and scales.

When the fermentation is completed, the culture broth is then subjected for recovery of the FR225497 substance to various procedures conventionally used for recovery and purification of biological active substance, for instance, solvent extraction with an appropriate solvent or a mixture of some solvents, chromatography or recrystallization from an appropriate solvent or a mixture thereof.

The FR225497 substance as obtained has the following physico-chemical properties:

Appearance:
white powder

Molecular formula :
$C_{34}H_{44}N_4O_5$

Elementary Analysis:
Calcd for $C_{34}H_{44}N_4O_5 \cdot 1/2\ H_2O$
C 68.32, H 7.59, N 9.35
Found:
C 68.61, H 7.52, N 9.37

Molecular weight:
ESI-MS (+): m/z 589 $(M + H)^+$
ESI-MS (-): m/z 587 $(M - H)^-$

Melting point:
193-194 °C

Specific rotation:
$[\alpha]^D$(23°C) -64 ° (c=0.5, in chloroform)

Ultraviolet absorption spectrum:
λmax (methanol): 235 (sh) nm
λmax (methanol+NaOH): 235 (sh) nm

Solubility:

Soluble: methanol, chloroform, ethyl acetate

Insoluble: water, n-hexane

Color reaction:

Positive: ceric sulfate reaction, iodine vapor reaction

Negative: ninhydrin reaction, ferric chloride reaction, Molish reaction, Ehrlich reaction, Dragendorff reaction

Thin layer chromatography (TLC):

Stationary phase	Developing solvent	Rf value
Silica Gel 60 F254*	chloroform:methanol (20:1, v/v)	0.66

* made by E. Merck

High Performance Liquid Chromatography (HPLC):

Condition:

Mobile phase: acetone:water=50:50

Column: YMC ODS AM-303** (250 x 4.6 mm i. d.)

Flow rate: 1.0 ml/minute

Detection: UV at 210 nm

Retention time: 15.3 minutes

** trade name: made by Yamamura Chemical Institute

Infrared Spectrum:

νmax (KBr): 3340, 3320, 2930, 1710, 1690, 1670, 1660, 1630, 1530, 1510, 1420, 1310, 1170, 1110 cm^{-1}

^1H Nuclear magnetic resonance spectrum:

(500 MHz, $CDCl_3$) δ_H

7.45 (1H, d, J=10Hz, exchangeable), 7.32 - 7.18 (8H, m), 7.12 - 7.09 (2H, m), 6.44 (1H, d, J=10Hz, exchangeable), 6.37 (1H, d, J=6Hz, exchangeable), 5.35 (1H, m), 5.01 (1H, m), 4.16 (1H, m), 3.94 (1H, m), 3.75 - 3.63 (2H, m), 3.28 - 3.18 (2H, m), 3.10 - 2.98 (2H, m), 2.43 - 2.34 (4H, m), 2.08 (1H, m), 1.97 (1H, m), 1.78 (1H, m), 1.72 (1H, m), 1.54 - 1.47 (5H, m), 1.32 - 1.16 (5H, m), 1.06 (3H, t, J=7Hz)

^{13}C Nuclear magnetic resonance spectrum:

(125 MHz, $CDCl_3$) δ_C

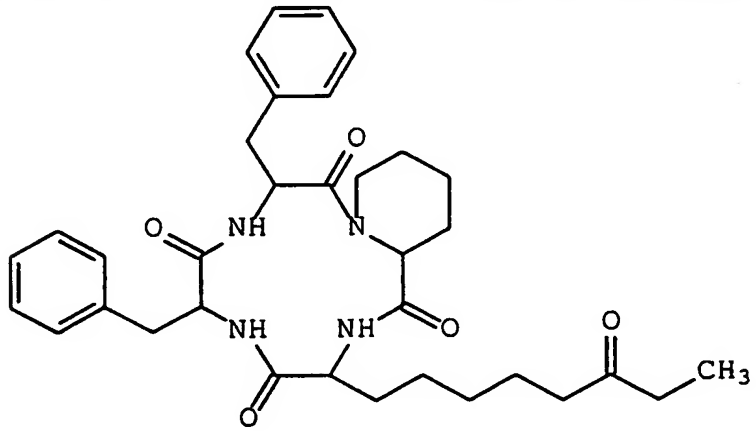
211.5 (s), 175.8 (s), 173.6 (s), 173.3 (s), 171.4 (s), 137.0 (s), 137.0 (s), 129.1 (d)

x2, 128.9 (d) x2, 128.6 (d) x2, 128.5 (d), x2, 126.9 (d), 126.7 (d), 62.9 (d), 53.5 (d), 50.9 (d), 50.0 (d), 43.9 (t), 42.1 (t), 36.5 (t), 35.9 (t), 35.2 (t), 29.0 (t), 28.7 (t), 25.2 (t), 25.1 (t), 24.0 (t), 23.5 (t), 19.2 (t), 7.8 (q)

Nature:

Neutral substance

From the above physico-chemical properties and extensive studies, the chemical structure of FR225497 substance was assigned as follows.



(I)

FR225497 substance can be in a form of a solvate, which is included within the scope of the present invention. The solvate preferably include a hydrate and an ethanolate.

As examples for showing biological activities of the FR225497 substance, some biological data are explained in the following.

Test 1: Effect of FR225497 substance on lymphocyte blastogenic response

The lymphocyte blastogenesis test was performed in microtiter plates with each well containing 1 x 10^5 splenic cells of Balb/c mice in 0.1 ml RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS), 50mM 2-mercaptoethanol, penicillin (100 units/ml) and streptomycin (100 μg/ml), to which anti-CD3 antibody (2C11) (1 μg/ml) was added. The cells were incubated at 37°C in a humidified atmosphere of 5% CO_2 for 72 hours. After the culture period, suppressive activities of the test samples in lymphocyte blastogenesis

were quantified by a MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide] dye reduction assay.

FR225497 substance was dissolved in methanol and further diluted in RPMI- 1640 medium and added to the culture to give final concentrations of 1250 ng/ml or less.

The results are shown in Table 1.

As shown in Table 1, FR225497 substance suppressed murine lymphocyte blastogenesis induced by anti- CD3 antibody in a dose-dependent manner.

Table 1 Effect of FR225497 substance on murine lymphocyte blastogenesis induced by anti-CD3 antibody

Concentration (ng/ml)	1250	625	313	156	78	39	20	10
Inhibition (%)	116.2	116.6	117.3	115.7	96.1	44.8	3.9	1.6

Test 2: Effect of FR225497 substance on DTH (delayed type hypersensitivity) response in mice

Female Balb/c mice were immunized with sheep red blood cells (1×10^8) by subcutaneous injection. FR225497 substance was dissolved in olive oil and administered subcutaneously for 8 consecutive days, begining at one day before the immunization. Six days after the immunization, sheep red blood cells (1.25×10^8) were injected into right rear foot pad, and 24 hours later, footpad swelling was measured with dial guage (Ozaki MFG Co.,LTD.). The magnitude of the DTH was expressed as the thickness of the challenged right footpad as compared with the untreated left footpad.

As shown in Table 2, the footpad swelling was markedly suppressed by the administration of FR225497 substance in a dose-dependent manner without any body weight loss.

Table 2 Effect of FR225497 substance on DTH response in mice

	N	Dose (mg/kg)	Foot pad swelling (% Inhibition)	Body weight gain (g)
unprimed	5		100***	2.3±0.3***
primed (olive oil)	10		0	3.4±0.1
FR225497	5	0.1	32**	2.5±0.1***
substance	5	1.0	46***	3.8±0.3
	5	10	61***	4.0±0.3
			**: P<0.01	*** : P<0.001

Test 3: Incubation of FR225497 substance with rat liver S-9 fraction

A solution of 1 mg/ml of FR225497 substance was prepared in methanol . Ten μl of the solution was added to 990 μl of the solution of the rat liver S-9 fraction (0.8 mg protein/ml ; Oriental Yeast Co., LTD.) containing cofactors ($MgCl_2 \cdot 6H_2O$: 8 μmol, KCl : 33 μmol, glucose 6-phosphate : 5 μmol, NADPH : 4 μmol, NADH : 4 μmol and $Na_2HPO_4 \cdot 12H_2O$ / $NaH_2PO_4 \cdot 2H_2O$: 100 μmol ; Oriental Yeast Co.,LTD.) in a test tube (1.5 ml tube ; BIO-BIC). The solution mixture in the test tube was incubated for 30 minutes at 37℃ in a shaking bath. Appropriate blanks without S-9 protein and cofactors were incubated in the same manner.

After the incubation, 300 μl of the solution mixture was placed in a screw-cap test tube, and the reaction was terminated by the addition of 300 μl of methanol and 1500 μl of phosphate-buffered saline. After mixing vigorously for 10 sec., the remaining FR225497 substance was extracted with 3000 ml of ethyl acetate, 2000 μl of organic phase was placed in another test tube, and the ethyl acetate gently evaporated with a stream of nitrogen. Two-hundred μl of methanol was added, and the concentration of the FR225497 substance was analyzed by analytical HPLC indicated below. Trapoxin A and Trapoxin B were also tested in the same manner.

As shown in Table 3, compared with Trapoxin A and Trapoxin B, FR225497 substance was markedly stable at the incubation with S-9 protein and cofactors.

Table 3 Incubation of Trapoxin A, Trapoxin B and FR225497 substance with rat liver S-9 fraction

Remaining (%)		
Trapoxin A	Trapoxin B	FR225497 substance
0	3.3	95.2

(analytical HPLC condition)

column	YMC Pack ODS-AM AM303 , S-5 120A (250 mm L. x 4.6 mm ID, YMC Co., Ltd.)
eluent	50% aqueous acetonitrile
flow rate	1 ml/minute.
detection	UV at 210 nm
retention time	FR225497 substance 12.3 minutes.

Test 4: Effect of FR225497 substance on activity of partially purified human histone deacetylase

The partial purification of human histone deacetylase, the preparation of [3H] acetyl histones, and the assay for histone deacetylase activity were basically according to the method as proposed by Yoshida et al. (Yoshida, M. et al.: J. Biol. Chem., 265:17174-17179, 1900) as follows.

Partial Purification of Human Histone Deacetylase:

The human histone deacetylase was partially purified from human T cell leukemia Jurkat cells. Jurkat cells (5×10^8 cells) were suspended in 40 ml of the HDA buffer consisting of 15 mM potassium phosphate, pH 7.5, 5% glycerol and 0.2 mM EDTA. After homogenization, nuclei were collected by centrifugation (35,000 x g, 10 minutes) and homogenized in 20 ml of the same buffer supplemented with 1 M $(NH_4)_2SO_4$. The viscous homogenate was sonicated and clarified by centrifugation (35,000 x g, 10 minutes), and the deacetylase was precipitated by raising the concentration of $(NH_4)_2SO_4$ to 3.5 M. The precipitated protein was dissolved in 10 ml of the HDA buffer and dialyzed

against 4 liters of the same buffer. The dialyzate was then loaded onto a DEAE-cellulose (Whatman DE52) column (25 x 85 mm) equilibrated with the same buffer and eluted with 300 ml a linear gradient (0-0.6 M) of NaCl. A single peak of histone deacetylase activity was eluted between 0.3 and 0.4 M NaCl.

Preparation of [^{3}H] Acetyl Histones:

To obtain [^{3}H]acetyl-labeled histones as the substrate for the histone deacetylase assay, 1 x 10^8 cells of Jurkat in 20 ml of RPMI-1640 medium supplemented with 10% FBS, penicillin (50 units/ml) and streptomycin (50 μg/ml) were incubated with 300 MBq [^{3}H] sodium acetate in the presence of 5 mM sodium butyrate for 30 minutes in 5% CO_2-95% air atmosphere at 37℃ in a 75 cm^2 flask, harvested into a centrifuge tube (50 ml), collected by centrifugation at 1000 rpm for 10 minutes, and washed once with phosphate-buffered saline. The washed cells were suspended in 15 ml of ice-cold lysis buffer (10 mM Tris-HCl, 50 mM sodium bisulfite, 1% Triton X-100, 10 mM $MgCl_2$, 8.6% sucrose, pH 6.5). After Dounce homogenization (30 stroke), the nuclei were collected by centrifugation at 1000 rpm for 10 minutes, washed three times with 15 ml of the lysis buffer, and once with 15 ml of ice-cold washing buffer (10 mM Tris-HCl, 13 mM EDTA, pH 7.4) successively. The pellet was suspended in 6 ml of ice-cold water using a mixer, and 68 ml of H_2SO_4 was added to the suspension to give a concentration of 0.4 N. After incubation at 4℃ for 1 hour, the suspension was centrifuged for 5 minutes at 15,000 rpm, and the supernatant was taken and mixed with 60 ml of acetone. After overnight incubation at -20℃, the coagulated material was collected by microcentrifugation, air-dried, and stored at -80℃.

Assay for Histone Deacetylase Activity:

For the standard assay, 10 μl of [^{3}H]acetyl-labeled histones were added to 90 μl of the enzyme fraction, and the mixture was incubated at 25℃ for 30 minutes. The reaction was stopped by addition of 10 μl of HCl. The released [^{3}H]acetic acid was extracted with 1 ml of ethyl acetate, and 0.9 ml of the solvent layer was taken into 10 ml of toluene scintillation solution for determination of radioactivity.

As shown in Table 4, FR225497 substance potently inhibited the activity of partially purified human (Jurkat cells) histone deacetylase in a dose-dependent manner.

Table 4 Effect of FR225497 substance on activity of partially purified human histone deacetylase

Concentration (ng/ml)	1000	100	10	1
Inhibition (%)	91.7	67.5	33.4	8.12

Test 5: Antitumor activities of FR225497 substance against human tumor cell lines

The cytotoxic activity of FR225497 substance against human tumor cell lines in vitro was determined as follows. Concentration of the compound required for 50% inhibition of cell growth (IC50; ng/ml) was examined by plotting the logarithms of the concentration vs. the growth rate (percentage of control) of the treated cells. Human T cell leukemia Jurkat cells (1×10^5 cells/ml) and human colon adenocarcinoma HT-29 cells (5×10^4 cells/ml) were treated with FR225497 substance in 100 µl of RPMI-1640 medium supplemented with 10% FBS, penicillin (50 units/ml) and streptomycin(50 µg/ml) in 5% CO_2-95% air atmosphere at 37℃. The cytotoxity was colorimetrically determined at 550 nm (and 660 nm as a reference) according to the MTT method described above.

The result was shown in Table 5. FR225497 substance had potent antitumor activities against Jurkat cells and HT-29 cells.

Table 5 Antitumor activities of FR225497 substance against human tumor cell lines (in vitro)

IC_{50} (ng/ml)	
Jurkat	HT-29
152	158

The pharmaceutical composition of this invention comprising FR225497 is useful as a therapeutic or prophylactic agent for diseases caused by abnormal gene expression such as inflammatory disorders, diabetes, diabetic complications, homozygous thalassemia, fibrosis, cirrhosis, acute promyelocytic leukemia (APL), protozoal infection or the like. Further, it is useful as an antitumor agent and immunosuppressant, which prevent an organ transplant rejection and autoimmune diseases as exemplified below.

Rejection reactions by transplantation of organs or tissues such as the heart, kidney, liver, bone marrow, skin, cornea, lung, pancreas, small intestine, limb, muscle, nerve, intervertebral disc, trachea, myoblast, cartilage, etc.; graft-versus-host reactions following bone marrow transplantation; autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus, Hashimoto's thyroiditis, multiple sclerosis, myasthenia gravis, type I diabetes, etc.; and infections caused by pathogenic microorganisms (e.g. Aspergillus fumigatus, Fusarium oxysporum, Trichophyton asteroides, etc.).

Furthermore, pharmaceutical preparations of FR225497 are useful for the therapy and prophylaxis of the following diseases.

Inflammatory or hyperproliferative skin diseases or cutaneous manifestations of immunologically-mediated diseases (e.g. psoriasis, atopic dermatitis, contact dermatitis, eczematoid dermatitis, seborrheic dermatitis, lichen planus, pemphigus, bullous pemphigoid, epidermolysis bullosa, urticaria, angioedema, vasculitides, erythema, dermal eosinophilia, lupus erythematosus, acne, and alopecia areata); autoimmune diseases of the eye (e.g. keratoconjunctivitis, vernal conjunctivitis, uveitis associated with Behcet's disease, keratitis, herpetic keratitis, conical keratitis, corneal epithelial dystrophy, keratoleukoma, ocular premphigus, Mooren's ulcer, scleritis, Graves' ophthalmopathy, Vogt-Koyanagi-Harada syndrome, keratoconjunctivitis sicca (dry eye), phlyctenule, iridocyclitis, sarcoidosis, endocrine ophthalmopathy, etc.);
reversible obstructive airways diseases [asthma (e.g. bronchial asthma, allergic asthma, intrinsic asthma, extrinsic asthma, and dust asthma), particularly chronic or inveterate asthma (e.g. late asthma and airway hyper-responsiveness)

bronchitis, etc.];
mucosal or vascular inflammations (e.g. gastric ulcer, ischemic or thrombotic vascular injury, ischemic bowel diseases, enteritis, necrotizing enterocolitis, intestinal damages associated with thermal burns, leukotriene B4-mediated diseases);
intestinal inflammations/allergies (e.g. coeliac diseases, proctitis, eosinophilic gastroenteritis, mastocyiosis, Crohn's disease and ulcerative colitis);
food-related allergic diseases with symptomatic manifestation remote from the gastrointestinal tract (e.g. migraine, rhinitis and eczema);
renal diseases (e.g. intestitial nephritis, Goodpasture's syndrome, hemolytic uremic syndrome, and diabetic nephropathy);
nervous diseases (e.g. multiple myositis, Guillain-Barre syndrome, Meniere's disease, multiple neuritis, solitary neuritis, cerebral infarction, Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis (ALS), and radiculopathy);
cerebral ischemic diseases (e.g., head injury, hemorrhage in brain (e.g., subarachnoid hemorrhage, intracerebral hemorrhage), cerebral thrombosis, cerebral embolism, cardiac arrest, stroke, transient ischemic attack (TIA), and hypertensive encephalopathy);
endocrine diseases (e.g. hyperthyroidism, and Basedow's disease);
hepatic diseases (e.g. pure red cell aplasia, aplastic anemia, hypoplastic anemia, idiopathic thrombocytopenic purpura, autoimmune hemolytic anemia, agranulocytosis, pernicious anemia, megaloblastic anemia, and anerythroplasia);
bone diseases (e.g. osteoporosis);
respiratory diseases (e.g. sarcoidosis, pulmonary fibrosis, and idiopathic interstitial pneumonia);
skin diseases (e.g. dermatomyositis, leukoderma vulgaris, ichthyosis valgaris, photosensitivity, and cutaneous T-cell lymphoma);
circulatory diseases (e.g. arteriosclerosis, atherosclerosis, aortitis syndrome, polyarteritis nodosa, and myocardosis);
collagen diseases (e.g. scleroderma, Wegener's granuloma, and Sjogren's syndrome);
adiposis;
eosinophilic fasciitis;

periodontal diseases (e.g. damage to gingiva, periodontium, alveolar bone or substantia ossea dentis);
nephrotic syndrome (e.g. glomerulonephritis);
male pattern alopecia, alopecia senile;
muscular dystrophy;
pyoderma and Sezary syndrome;
chromosome abnormality-associated diseases (e.g. Down's syndrome);
Addison's disease;
active oxygen-mediated diseases [e.g. organ injury (e.g. ischemic circulation disorders of organs (e.g. heart, liver, kidney, digestive tract, etc.) associated with preservation, transplantation, or ischemic diseases (e.g. thrombosis, cardial infarction, etc.)):
intestinal diseases (e.g. endotoxin shock, pseudomembranous colitis, and drug- or radiation-induced colitis);
renal diseases (e.g. ischemic acute renal insufficiency, chronic renal failure);
pulmonary diseases (e.g. toxicosis caused by pulmonary oxygen or drugs (e.g. paracort, bleomycin, etc.), lung cancer, and pulmonary emphysema);
ocular diseases (e.g. cataracta, iron-storage disease (siderosis bulbi), retinitis, pigmentosa, senile plaques, vitreous scarring, corneal alkali burn);
dermatitis (e.g. erythema multiforme, linear immunoglobulin A bullous dermatitis, cement dermatitis);
and other diseases (e.g. gingivitis, periodontitis, sepsis, pancreatitis, and diseases caused by environmental pollution (e.g. air pollution), aging, carcinogen, metastasis of carcinoma, and hypobaropathy)];
diseases caused by histamine release or leukotriene C4 release;
restenosis of coronary artery following angioplasty and prevention of postsurgical adhesions;
Autoimmune diseases and inflammatory conditions (e.g., primary mucosal edema, autoimmune atrophic gastritis, premature menopause, male sterility, juvenile diabetes mellitus, pemphigus vulgaris, pemphigoid, sympathetic ophthalmitis, lens-induced uveitis, idiopathic leukopenia, active chronic hepatitis, idiopathic cirrhosis, discoid lupus erythematosus, autoimmune orchitis, arthritis (e.g. arthritis deformans), or polychondritis);
Human Immunodeficiency Virus (HIV) infection, AIDS;

allergic conjunctivitis;
hypertrophic cicatrix and keloid due to traunra, burn, or surgery.

Therefore, the pharmaceutical composition of the present invention is useful for the therapy and prophylaxis of liver diseases [e.g. immunogenic diseases (e.g. chronic autoimmune liver diseases such as autoimmune hepatic diseases, primary biliary cirrhosis or sclerosing cholangitis), partial liver resection, acute liver necrosis (e.g. necrosis caused by toxins, viral hepatitis, shock, or anoxia), hepatitis B, non-A non-B hepatitis, hepatocirrhosis, and hepatic failure (e.g. fulminant hepatitis, late-onset hepatitis and "acute-on-chronic" liver failure (acute liver failure on chronic liver diseases))].

The pharmaceutical composition of this invention can be used in the form of pharmaceutical preparation, for example, in solid, semisolid or liquid form, which contains the FR225497, as an active ingredient in admixture with an organic or inorganic carrier or excipient suitable for external, enteral or parenteral administrations. The active ingredient may be compounded, for example, with the usual non-toxic, pharmaceutically acceptable carriers for tablets, pellets, capsules, suppositories, solutions, emulsions, suspensions, injections, ointments, liniments, eye drops, lotion, gel, cream, and any other form suitable for use.

The carriers which can be used are water, glucose, lactose, gum acacia, gelatin, mannitol, starch paste, magnesium trisilicate, talc, corn starch, keratin, colloidal silica, potato starch, urea and other carriers suitable for use in manufacturing preparations, in solid, semisolid, or liquid form, and in addition auxiliary, stabilizing, thickening, solubilizing and coloring agents and perfumes may be used.

For applying the composition to human, it is preferable to apply it by intravenous, intramuscular, topical or oral administration. While the dosage of therapeutically effective amount of the FR225497 substance varies from and also depends upon the age and condition of each individual patient to be treated, in the case of individual patient to be treated, in the case of intravenous

administration, a daily dose of 0.01 - 10 mg of the FR225497 substance per kg weight of human being, in the case of intramuscular administration, a daily dose of 0.1 - 10 mg of the FR225497 substance per kg weight of human being, in case of oral administration, a daily dose of 0.5 - 50 mg of the FR225497 substance of human being is generally given for treating.

THE BEST MODE OF CARRYING OUT OF THE INVENTION

Following examples are given for the purpose of illustrating the present invention in more detail.

EXAMPLE

(1) Fermentation of FR225497 substance :

An aqueous seed medium (160 ml) containing 1.0% polypeptone, 2.0% glucose, 0.3% beef extract, 0.2% yeast extract, 0.1% NaCl (pH 7.0) was poured into a 500-ml Erlenmeyer flask and sterilized at 120℃ for 30 minutes. A slant culture (POTETO DEXTROSE AGER (Difco 0013)) of the strain Helicoma ambiens RF-1023-1 was inoculated into the flask and cultured at 28℃ on a rotary shaker at 120 rpm (7 cm stroke) for 5-6 days. The seed culture (800 ml; 5 flasks) was inoculated to 20 liters of sterile production medium consisting of 2.0% potato starch, 2.0% sucrose, 0.5% yeast extract, 0.05% Adekanol LG-109 (defoaming agent, Asahi Denka Co., Ltd.) and 0.05% Silicone KM-70 (defoaming agent, Shin-Etsu Chemical Co., Ltd.) (pH 7.0) in a 30-liter jar fermentor. Fermentation was carried out at 25℃ for 14 days under aeration of 20 liters/minutes and agitation of 150 rpm.

The production of active compound in the fermentation broth was monitored by HPLC analysis.

(analytical HPLC condition)

column	YMC Pack ODS-AM AM303 , S-5 120A (250mm L. x 4.6 mm I.D., YMC Co., Ltd.)
eluent	50% aqueous acetonitrile
flow rate	1 ml/minute.

detection	UV at 210 nm	
retention time	FR225497 substance	12.3 minutes.

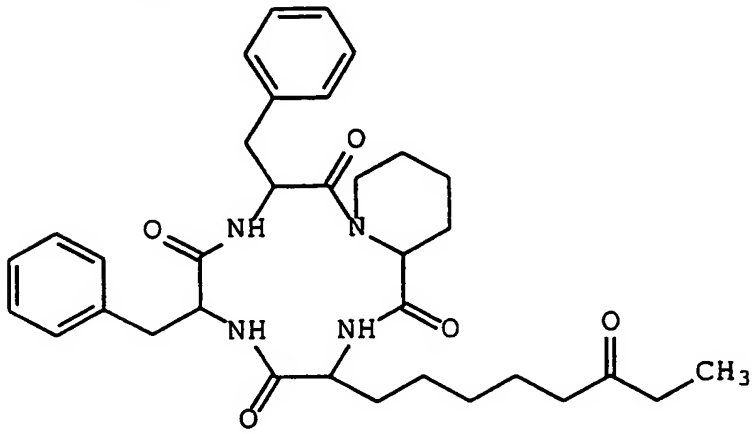
(2) Isolation of FR225497 substance :

The cultured broth (75L: containing 736 mg of FR225497 substance) was extracted with 75L of acetone by intermittent mixing. The acetone extract was filtered with an aid of diatomaceous earth and diluted with the same volume of water. The diluted filtrate was passed through a column (15L) of Diaion HP-20 (Mistubishi Chemical Co., Ltd.). The column was washed with water and 50% aqueous methanol, and eluted with methanol. The eluate (90L) was concentrated in vacuo to give an oily residue (containing 506 mg of FR225497 substance) .

The oily residue was dissolved in small volume of methanol and applied on a column (4L) of YMC GEL ODS-AM 120-S50 (YMC Co., Ltd.) packed with 50% aqueous acetonitrile. The column was eluted with 50% aqueous acetonitrile and elution was monitored by analytical HPLC indicated above. The portion corresponding to the purified FR225497 substance was concentrated in vacuo to give an aqueous residue. This residue was extracted with ethyl acetate and the extracts were concentrated in vacuo to give 270 mg of the purified FR225497 substance as a white powder.

CLAIMS

1. A compound of the formula (I):



(I)

2. A compound, having the following characteristics :

a) Molecular formula : $C_{34}H_{44}N_4O_5$

b) Melting point : 193-194°C

c) Solubility :

Soluble : Methanol, Chloroform, Ethyl acetate

Insoluble : Water, n-Hexane

d) Color reaction :

Positive : Ceric sulfate reaction, iodine vapor reaction

Negative : Ninhydrin reaction, ferric chloride reaction, Molish reaction, Erlich reaction, Dragendorff reaction

e) Infrared spectrum :

vmax(KBr) : 3340, 3320, 2930, 1710, 1690, 1670, 1660, 1630, 1530, 1510, 1420, 1310, 1170, 1110 cm^{-1}

3. A process for production of the compound of claim 1 or 2, which comprises culturing, in a nutrient medium, a FR225497 substance-producing strain belonging to Helicoma and recovering the compound of claim 1 or 2 from a culture broth.

4. A pharmaceutical composition containing the compound of claim 1 or 2, an active ingredient, in association with a pharmaceutically acceptable,

substantially non-toxic carrier or excipient.

5. A compound of claim 1 or 2 for use as a medicament.

6. A use of the compound of claim 1 or 2 for manufacture of a medicament for inhibiting histone deacetylase.

7. A use of the compound of claim 1 or 2 for manufacture of a medicament for treating or preventing inflammatory disorders, diabetes, diabetic complications, homozygous thalassemia, fibrosis, cirrhosis, acute promyelocytic leukaemia (APL), protozoal infection, organ transplant rejection, autoimmune diseases, or tumor.

8. A use of histone deacetylase inhibitors for manufacture of an immunosuppressant or an antitumor agent.

9. A use of histone deacetylase inhibitors for manufacture of a medicament for treating or preventing organ transplant rejection, autoimmune diseases, or tumor.